Metabolic responses to temperature stress under elevated pCO2 in Crepidula fornicata

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Title: METABOLIC RESPONSES TO TEMPERATURE STRESS UNDER ELEVATED pCO_2 IN THE SLIPPER LIMPET *CREPIDULA FORNICATA*

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Short running head: *C. fornicata* respiration under high pCO_2

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ABSTRACT

In the current context of environmental change, ocean acidification is predicted to affect the cellular processes, physiology and behavior of all marine organisms, impacting survival, growth and reproduction. In relation to thermal tolerance limits, the effects of elevated pCO_2 could be expected to be more pronounced at the upper limits of the thermal tolerance window. Our study focused on *Crepidula fornicata*, an invasive gastropod which colonized shallow waters around European coasts during the 20th century. We investigated the effects of 10 weeks' exposure to current (380 µatm) and elevated (550, 750, 1000 µatm) pCO_2 on this engineer species using an acute temperature increase (1°C 12h^{-1}) as the test. Respiration rates were measured on both males (small individuals) and females (large individuals). Mortality increased suddenly from 34°C, particularly in females. Respiration rate in *C. fornicata* increased linearly with temperature between 18°C and 34°C, but no differences were detected between the different pCO_2 conditions either in the regressions between respiration rate and temperature, or in Q_{10} values. In the same way, condition indices were similar in all the pCO_2 treatments at the end of the experiment but decreased from the beginning of the experiment. This species was highly resistant to acute exposure to high temperature regardless of pCO_2 levels, even though food was limited during the experiment. *C. fornicata* appears to have either developed resistance mechanisms or a strong phenotypic plasticity to deal with fluctuations of physico-chemical parameters in their habitat. This suggests that this invasive species may be more resistant to future environmental changes compared to its native competitors.

Keywords: CO_2 stress, invasive species, ocean acidification, Q_{10}, respiration, temperate waters

INTRODUCTION

As part of global change, ocean acidification is caused by increasing anthropogenic CO_2 emissions which have increased since the beginning of the industrial revolution (Solomon *et al.*, 2007). Future pCO_2 increases are predicted to reduce the pH of surface waters by 0.3 - 0.4 units by the end of the century (Caldeira & Wickett, 2003). Such decreases will produce changes in carbon and carbonate seawater chemistry through decreased carbonate ion concentrations (CO_3^{2-}) and a lower calcium carbonate saturation state (Ω). These changes are predicted to have major consequences for marine life (Fabry *et al.*, 2008; Kroeker *et al.*, 2013b) and, especially, could have broad impacts on physiological functions of heterotrophic marine organisms (Pörtner, 2008; Hofmann & Todgham, 2010).

The decrease in pH is likely to have a wide range of effects on marine invertebrates via shifts in acid-base homeostasis, changes in metabolism and energy balance (Pörtner *et al.*, 2005), leading to effects on somatic growth (Berge *et al.*, 2006; Thomsen & Melzner, 2010), respiration (Melatunan *et al.*, 2011; Schalkhausser *et al.*, 2013), excretion (Liu & He, 2012), calcification (Gazeau *et al.*, 2007; Wood *et al.*, 2008; Watson *et al.*, 2012) or feeding rates (Bamber, 1990; Navarro *et al.*, 2013). Many marine invertebrates exposed to elevated pCO_2 have exhibited metabolic depression (Willson & Burnett, 2000; Michaelidis *et al.*, 2005; Navarro *et al.*, 2013) as a decrease in respiration rate while others have remained unaffected (Gutowska *et al.*, 2008; Lannig *et al.*, 2010; Clark *et al.*, 2013) or even increased their metabolic rate (Wood *et al.*, 2008; Beniash *et al.*, 2010). These responses are highly species-specific and may vary with organism size (Beniash *et al.*, 2010). The resilience of the species studied, and the capacity to regulate metabolism under stressful conditions are also important (Pörtner, 2008). These physiological impacts are likely to have broad effects on the survival, growth and reproduction of marine species (Shirayama & Thornton, 2005; Byrne, 2011),

which would lead to changes in community structure from altered diversity and abundances (Hale *et al*., 2011; Kroeker *et al*., 2013a).

These physiological impacts are likely modulated by temperature because temperature is a primary driver of physiological function in ectotherms (Hofmann & Todgham, 2010). Increasing temperature affects the rate of all biochemical reactions, and hence cellular processes and physiological functions (Clarke, 1983; Pörtner, 2012), increasing metabolic costs within a limited thermal tolerance window (Peck *et al*., 2002; Marshall *et al*., 2003). The interactive effects of increased temperature and elevated CO_2 concentrations are predicted to impair physiological processes (Clarke, 2003; Pörtner, 2008) by narrowing the thermal tolerance window of the organisms (Metzger et al., 2007; Lannig et al., 2010) and elevating vulnerability to extreme temperature (Schalkhausser et al., 2012).

In a context of global change, non-indigenous species are expected to be favored in their introduced area (Dukes & Mooney, 1999; Occhipinti-Ambrogi, 2007) mainly because robustness to abiotic variation is often a trait that determines the success of invasive of a species (Hellmann *et al*., 2008; Lenz *et al*., 2011). Climatic changes in the physical environment will likely affect the distribution, spread, abundance, impacts and interactions of species, possibly to the advantage of introduced organisms (Occhipinti-Ambrogi, 2007). Thus our study focused on the response of an invasive Calyptraeidae gastropod living on western European coasts, but which originates from North East America. The slipper limpet, *Crepidula fornicata* (Linné 1758) was introduced in Europe at the end of the 19th century, mainly with oysters (*Crassostrea gigas*) which were imported for farming (Blanchard, 1995), and has subsequently colonized European coasts from southern Sweden to southern France (Blanchard, 1997). *C. fornicata* has significant impacts on biodiversity and ecosystem functioning where it has established (De Montaudouin *et al*., 1999; Decottignies *et al*., 2007; Martin *et al*., 2007). It lives in shallow sites, especially in bays and estuaries where very high

densities of over one thousand individuals m^{-2} have been reported (Blanchard, 1995). *C. fornicata* is known to be strongly resistant to environmental variations, particularly temperature and salinity (Blanchard, 1995; Blanchard, 1997; Diederich & Pechenick, 2013). In light of the different ecological and physiological characteristics of *C. fornicata*, it is important to investigate the impact of future pCO_2 levels, and determine its resistance capacities to high levels of stress to assess the likely future impact of this engineer species in the ecosystems to which it was introduced.

The present study was designed to investigate the metabolic responses of *C. fornica*ta to high pCO_2 conditions during temperature stress. Short-term experimental approaches using faster temperature elevations than natural changes provide valuable insight into physiological responses of marine invertebrates in term of their ability to resist high levels of stress or their lethal temperature (Sokolova & Pörtner, 2003; Peck *et al*., 2004; Pörtner *et al*., 2006; Richard *et al*., 2012). Following the hypothesis that CO_2 stress will increase sensitivity to temperature change, we evaluated changes in oxygen-consumption of *C. fornicata* individuals previously reared under elevated pCO_2 for 10 weeks during a rapid temperature increase (1°C $12h^{-1}$). Respiration rates were measured as a proxy for metabolism on males (small individuals) and females (large individuals), as in this species there is sexual dimorphism in size.

FMATERIAL & METHODS

Biological material

Crepidula fornicata stacks were collected by SCUBA divers on 4 February 2010, in Morlaix Bay (northwest Brittany, France), at the "Barre des Flots" site (3°53.015'W; 48°40.015'N) at a depth of 10 meters and at an *in situ* temperature of 11.6°C (SOMLIT: *Service d'Observation de la Mer et du LITtoral* data). They were transferred directly to

aquaria at the Station Biologique de Roscoff where they were held in natural unfiltered seawater at a temperature around 10°C, until they were used in experiments starting on 10 March 2010.

Males and females at the top and the bottom of stacks respectively, were selected, separated and individually labelled. Small males (23.31 ± 0.16 mm length), which were still slightly mobile, were placed individually on 3 cm Petri dishes one month before the beginning of the trials. Dead individual shells at the base of stacks were kept as the substratum under the largest living immobile females (47.53 ± 0.25 mm length). In *C. fornicata*, size cannot be discriminated from sex because this is a protandrous hermaphroditic organism, changing sex with age and size (Coe 1938). All individuals were gently brushed to remove epibionts and biofilm from their shells before proceeding to the metabolic measurements.

Condition indices (CI) were calculated on a pool of 20 specimens in March, before the beginning of the experiment, and on all remaining living and recently dead individuals (male n = 74; female n = 99) at the end of the temperature increase on 29 May 2010. Shell dry weight (DW_{Shell}), shell length and tissue dry weight (DW_{Tissue}) were determined separately on each individual after drying at 60°C for 48h. Specimens were then ignited in a muffle furnace at 520°C for 6 h, with tissue ash-free dry weight ($AFDW_{Tissue}$) being obtained by difference. CI were calculated as:

$$CI = (AFDW_{Tissue} / DW_{Shell}) \times 100.$$

Mortality was checked daily during the experiment. Individuals with no reaction when the foot was stimulated were classed as dead and removed from the tanks.

Experimental conditions and set-up

After distributing randomly in each of twelve 10-L aquarium tanks comprising the experimental flow-through system (as described in Noisette *et al*., 2013), 120 males and 120

158 females (i.e. 10 individuals of each sex per aquarium) were held in different pCO_2 conditions
159 between 13 March and 29 May 2010. At the beginning of the experiment, pH was gradually
160 decreased (by bubbling CO_2) over four days at 0.1 pH units day^{-1} from 8.1 until the required
161 pH was reached. Specimens were subsequently held for ten weeks in four different pCO_2
162 conditions: a current pCO_2 of 380 µatm (pH_T = 8.07), and three elevated pCO_2 levels of 550
163 µatm (pH_T = 7.94), 750 µatm (pH_T = 7.82) and 1000 µatm (pH_T = 7.77). The elevated pCO_2
164 values corresponded to different scenarios predicted by the Intergovernmental Panel on
165 Climate Change (IPCC) for the end of the century (Solomon *et al*., 2007) and were selected
166 according to the recommendations of Barry *et al.*, (2010). pCO_2 was adjusted by bubbling
167 CO_2-free air (current pCO_2) or pure CO_2 (elevated pCO_2) in four 100 L header tanks (1 per
168 pCO_2 condition) supplied with natural unfiltered seawater pumped from the sea, directly at the
169 foot of the Station Biologique de Roscoff. Seawater was continually delivered by gravity from
170 each header tank to three aquaria per pCO_2 condition at a constant rate of 9 L h^{-1} (renewal
171 rate: 90% total aquarium volume h^{-1}). pCO_2 was monitored and controlled by a feedback
172 system (IKS Aquastar, Karlsbad, Germany) that regulated the addition of gas in the header
173 tanks. pH values of the pH-stat system were adjusted from daily measurements of pH on the
174 total scale (pH_T) in the aquaria using a pH meter (HQ40D, Hach Lange, Ltd portable LDO™,
175 Loveland, Colorado, USA) calibrated using Tris/HCl and 2-aminopyridine/HCl buffers
176 (Dickson et al., 2007). The twelve aquaria were placed in four thermostatic baths where
177 temperature was controlled to ± 0.2 °C using 100 - 150 W submersible heaters.

178 Before the rapid temperature increase experiment, *C. fornicata* individuals were
179 maintained in the different pCO_2 treatments for 10 weeks while temperature was raised
180 successively to mimic the natural rate of temperature change from winter to summer.
181 Temperature was maintained at 10°C from the beginning of the trial to 29 March. It was
182 raised to 13°C from 5 to 19 April and to 16°C from 26 April to 18 May 2010. To reach these

183 set levels the temperature was increased by 0.5°C day^{-1} until the new set temperature was
184 achieved. During the experiment, animals were naturally fed by the phytoplankton provided
185 by unfiltered seawater.

186 The rapid temperature increase experiment was conducted between the 18 and 29 May
187 2010. In all four pCO_2 treatments, temperature was increased from 16 to 36°C at 1°C $12h^{-1}$. *C.*
188 *fornicata* oxygen consumption was measured (see below) both in small and large individuals
189 in the different pCO_2 treatments during this rapid temperature increase.

190

191 *Seawater parameters*

192 Seawater parameters were monitored throughout the experiment. pH_T and temperature
193 were recorded daily in each of the 12 aquaria using a pH meter (HQ40D, Hach Lange, Ltd
194 portable LDO™, Loveland, Colorado, USA). Total alkalinity was determined every 3 weeks
195 by 0.01N HCl potentiometric titration on an automatic titrator (Titroline alpha, Schott SI
196 Analytics, Mainz, Germany). Seawater carbonate chemistry, *i.e.* exact CO_2 partial pressure
197 (pCO_2) and saturation state of aragonite were calculated in each pCO_2 condition using
198 CO_2SYS software (Lewis & Wallace, 1998) using constants from Mehrbach *et al*., (1973)
199 refitted by Dickson & Millero, (1987). Mean values (± standard error, SE) of the parameters
200 in each pCO_2 treatment are presented in Table 1.

201

202 *Oxygen consumption measurements*

203 During the rapid temperature increase trial (18 - 29 May 2010), oxygen consumption
204 of 6 randomly selected labeled individuals of each sex (2 per aquaria) was measured in each
205 of the pCO_2 treatments every two days, at 18, 22, 26, 30 and 34°C. Respiration rates were
206 determined using closed incubations in 75 mL (males) or 180 mL (females) acrylic chambers
207 (Engineering & Design Plastics Ltd, Cambridge, UK) filled with water from the same

aquarium (see methods in Morley *et al*., 2007). Chambers were placed in their respective aquaria during incubations to keep the temperature constant. Incubations varied between 1 h and 3 h depending on temperature and were halted before oxygen saturation fell below 80% saturation. Control incubations without animals (n = 1 control incubation / aquarium / measurement) were carried out to allow correction for microbial activity in seawater.

Respiration rates were calculated from the differences in measurements of oxygen concentration during trials and controls using a non-invasive fiber-optical system (FIBOX 3, PreSens, Regensburg, Germany) made up of an optical fiber and reactive oxygen spots attached to the inner wall of the chambers. These spots were calibrated with 0% and 100% oxygen buffers made from the manufacturer instructions. 0% O_2 buffer was prepared by dissolving 10 g of Na_2SO_3 in 1 L of seawater and 100% O_2 buffer was prepared by bubbling air in 1L of seawater for 20 min to achieve oxygen saturation. Previous experiments had demonstrated that oxygen consumption remained linear during all the incubation periods. Chamber contents were mixed gently by inverting chambers several times before each oxygen measurement. Respiration (R) rates (in µmol O_2 g^{-1} AFDW h^{-1}) were corrected for oxygen consumption in controls and calculated as:

$$R = -(\Delta O_2 \times V) / (\Delta t \times AFDW_{Tissue})$$

where ΔO_2 (µmol O_2 L^{-1}) is the difference between initial and final O_2 concentrations during the incubation, V (L) is the chamber volume minus the individual *C. fornicata* volume, Δt (h) is the incubation time and $AFDW_{Tissue}$ (g) is the tissue ash free dry weight of the slipper limpet incubated.

Q_{10} coefficients were calculated by using the standard equation:

$$Q_{10} = (R_H / R_L)^{10 / (T_H - T_L)}$$

where T_L and T_H were the lowest and highest temperature reached and R_L and R_H the respiration rates in these temperature respectively.

Statistical analyses

All statistical analyses were performed using R version 2.15.0 (R Core Team 2013) and STATISTICA software. A logistic regression (general linear model, GLM) was applied to test the differences in mortalities between the different pCO_2 treatments and between sex with temperature as the linear variable. The effects of pCO_2, sex and the interaction of these two factors on condition index (CI) at the end of the experiment and on Q_{10} values were investigated by 2-way analysis of variance (ANOVA). Linear regressions between respiration rates and increasing temperatures were fitted in the four different pCO_2 treatments for males and females separately. Differences between pCO_2 treatments were explored using an ANCOVA with pCO_2 and sex as fixed factors and temperature as co-variable.. Normality was assessed using the Kolmogorov-Smirnov test and Levene's test was used to ensure that variances were homogenous. All the results are presented as mean ± standard error (SE).

RESULTS

Mortality occurred between 34 and 36°C for females and 22 and 36°C for males (Figure 1). There were no significant differences in mortality between the different pCO_2 treatments (GLM, df = 3, F = 0.680, p = 0.565) or between males and females (GLM, df = 1, F = 0.580, p = 0.449). Moreover, the interaction between factors pCO_2 and sex of the individuals was not significant (GLM, df = 3; F = 0.21; p = 0.888). At pCO_2 levels of 380, 550, 750 and 1000 µatm, the mortality was 29, 19, 19, and 24 for females and 28, 6, 8, and 6 for males . At the end of the acute temperature increase nearly twice the number of females had died (91) compared with the males (48) (χ^2 test, $p < 0.05$).

The mean condition index before the start of the experiment was 3.00 ± 0.27 (n=10). It varied at the end of the experiment between 1.69 ± 0.13 for males at pCO_2 of 380 µatm and 2.41 ± 0.27 for females at pCO_2 of 550 µatm (Table 2). There were no effects of pCO_2 , sex or the interaction of these two factors on the condition index at the end of the trial (Table 2). However, the condition index from the beginning of the experiment (3.00 ± 0.27) was different from the mean condition index including all pCO_2 conditions (2.11 ± 0.07) at the end of the trial (t-test, df = 181, t = 3.159, p = 0.002), which means that CI in both males and females decreased significantly from the start to the end of the experiment (Figure 2).

Female respiration rates varied between 0.51 µmol O_2 g^{-1} AFDW h^{-1} at 18°C and pCO_2 of 750 µatm and 91.62 µmol O_2 g^{-1} AFDW h^{-1} at 32°C and pCO_2 of 380 µatm. Males had higher rates, which ranged between 5.13 µmol O_2 g^{-1} AFDW h^{-1} at 18°C and pCO_2 of 380 µatm and 175.51 µmol O_2 g^{-1} AFDW h^{-1} at 32°C and pCO_2 of 380 (Figure 3).

Relationships between respiration rate and temperature were linear at each pCO_2 level (Figure 3). Respiration rose significantly with increasing temperature in all pCO_2 treatments, for both males and females (Table 3, all p-values < 0.02). There were no significant differences between the slopes of the different regressions among the pCO_2 treatments or between sexes (analysis of slopes, df = 3, F = 1.1, p = 0.346). The intercepts of the different regressions also did not significantly vary among pCO_2 (ANCOVA, df = 3, F = 0.350, p = 0.789), but there were difference between males and females (ANCOVA, df = 1, F = 62.63, p < 0.001).

Q_{10} values ranged from 1.24 to 2.40 for females and from 1.36 to 2.77 for males among the different pCO_2 treatments (Figure 2). There was no significant pCO_2 effect on Q_{10} values for either males or females (Table 2). Across all pCO_2 treatments, females had significantly lower Q_{10} values than males with means of 1.61 ± 0.11 and 2.00 ± 0.12 for

females and males, respectively (Table 2). The interaction between pCO_2 and sex, however, was not significant (Table 2).

DISCUSSION

Independently of the impact of pCO_2 we planned to test, one of the major issues of this study was food limitation which was unintentionally imposed on the *C. fornicata* individuals in the experiments. This food limitation was detected because the decrease in condition indices (CI) of both males and females from the beginning to the end of the experiment. Such decreases in CI are usually related to food quantity or quality supplied to organisms (Norkko & Thrush, 2006). Animals were maintained in unfiltered seawater which carried natural phytoplankton at a concentration between 0.2 and 1 µg Chl a L^{-1} (*SOMLIT* data). The water renewal in the aquarium was maintained constant at a rate of 0.9 L h-1 (i.e. 90% of the total volume of each aquarium changed per hour). Water supply in our experimental system was likely too low to provide sufficient food for the experimental animals, which thus relied on internal energy reserves and so decreased their CI. A similar outcome was reported for mussels by Mackenzie *et al.* (2014).

The use of stored reserves was similar in the different pCO_2 conditions as CI at the end of the experiment did not differ between the different pCO_2 treatments, and this was the case for both sexes. Previous studies have shown interspecific variability in the responses of condition indices under high pCO_2 levels, ranging from a lack of effect (Cummings *et al*., 2011; Clark *et al*., 2013; Sanders *et al*., 2013) to large changes in condition under high pCO_2 levels (Hiebenthal *et al*., 2013; Range *et al*., 2014). Energy availability is a major component in mitigating the effects of ocean acidification (Pansch *et al.*, 2014). Studies have shown that an abundant food supply might counteract even overcome the negative effects of high pCO_2

306 on adult and juvenile bivalves (Melzner *et al.*, 2011; Thomsen *et al.*, 2013). Thus, it is
307 important to consider that in this study *C. fornicata* were under limited food conditions when
308 interpreting their metabolic responses to elevated pCO_2 conditions during the temperature
309 rise. The data here are representative of conditions where there is temperature stress and food
310 supplies are limited, conditions that can occur in the field.

311 The limitation of food supply was not markedly more important in any of our reduced
312 pH conditions as there were no differences in mortality rates between the different pCO_2
313 treatments in *C. fornicata* males and females. This is a different outcome to that reported for
314 some other mollusk species held in elevated pCO_2 levels (Shirayama & Thornton, 2005;
315 Beniash *et al*., 2010). However, similarly to our study, Pansh *et al.*, (2014) showed that food
316 availability had no impact on mortalities of the barnacle *Amphibalanus improvises* held in
317 different pCO_2 conditions. In the present study, important mortalities started to occur from
318 32°C and they became larger at and above 34°C for both males and females. These values are
319 consistent with the upper lethal temperature recorded for *C. fornicata* by Diederich &
320 Pechenick, (2013) in a laboratory study investigating a population from Rhode Island, USA,
321 in which only 40% of the adults survived after a 3 h exposure to 34°C, and all died after a 3 h
322 exposure to 36°C. Mortality was higher in females (larger individuals) than in males (small
323 individuals) even if, male started to die at lower temperatures than females. Similarly, Peck *et*
324 *al.*, (2009) demonstrated for 14 species that smaller species survived to higher temperatures
325 than large ones when temperature was raised at 1°C day^{-1}, and Peck *et al.*, (2013) showed that
326 juveniles had higher upper temperature limits than adults in 4 species of marine invertebrates
327 at warming rates of 1°C day^{-1} and 1°C $3days^{-1}$. The mechanisms setting temperature limits at
328 acute rates of warming may not be energy availability (Peck *et al*., 2014) and females, which
329 had more energetic reserves than males, may thus have not had an advantage.

330 Despite the decreases in CI, mean respiration rates of *C. fornicata* at 18°C and pCO_2
331 of 380 µatm were 31 and 26 µmol O_2 g^{-1} AFDW h^{-1} for males and females, respectively,
332 which are close to the middle of the range of *in situ* values reported for wild individuals from
333 the Bay of Brest (Brittany, France) (6 to 63 µmol O_2 g^{-1} AFDW h^{-1}: Martin *et al*., 2006). This
334 indicates that animals in the experiments here had similar oxygen consumption than wild
335 specimens and were not metabolically depressed under insufficient food supply. In both *C.*
336 *fornicata* males and females, respiration rates increased with temperature, as previously
337 demonstrated for this species by Newell & Kofoed, (1977) and most ectotherm metabolic
338 rates are correlated positively with temperature (Cossins & Bowler, 1987). Respiration rates
339 were higher in *C. fornicata* males than in females regardless of the temperature. Generally,
340 mass-specific respiration rates of small individuals are higher than those of larger ones
341 because metabolic rate (normalized to the biomass) decreases with increasing organisms size
342 (von Bertalanffy, 1951; Parsons *et al*., 1984).
343 The relationship between oxygen consumption and temperature here for *C. fornicata*
344 was similar in all the different pCO_2 treatments. The slopes and intercepts of the regressions
345 were not significantly different across the four pCO_2 conditions which means temperature
346 effect on respiration rate was not affected by the different pCO_2 levels in males or females. In
347 constrast to our results, Lannig *et al*., (2010) found that an acute temperature rise
348 (1.25°C/12h) caused a more rapid increase in metabolic rate in *Crassostrea gigas* under
349 elevated pCO_2 conditions, and there was a synergistic effect of temperature and pCO_2. The
350 lack of difference in respiration between animals held in different pCO_2 conditions may be
351 related to a stronger ability to up-regulate their metabolism under a temperature stress
352 irrespective of pCO_2. Thus, under warming conditions, *C. fornicata* can generate sufficient
353 energy to cope with any effects of decreased pH (Wood *et al*., 2010). Q_{10} values were also
354 similar across pCO_2 treatments in both males and females and they were within the expected

355 range of values recorded for marine invertebrates (Branch *et al*., 1988; Marshall *et al*., 2003).
356 Even if *C. fornicata* individuals were food limited, their oxygen consumption remained
357 unaffected by elevated pCO_2. A similar lack of pCO_2 effect was reported for growth and shell
358 strength of the barnacle *A. improvisus* (Pansch *et al.*, 2014). In our study, the low food supply
359 did not appear to affect the resistance or resilience of *C. fornicata* to CO_2 stress.

360 Several studies investigating the response of mollusk respiration to elevated pCO_2
361 have demonstrated metabolic depression under high pCO_2 in both bivalves and gastropods
362 (Michaelidis *et al*., 2005; Bibby *et al*., 2007; Fernandez-Reiriz *et al*., 2011; Melatunan *et al*.,
363 2011; Liu & He, 2012; Navarro *et al*., 2013). Conversely, others observed no pCO_2 effect on
364 mollusk respiration and general metabolism (Gazeau *et al*., 2007; Marchant *et al*., 2010;
365 Fernandez-Reiriz *et al*., 2012; Clark *et al*., 2013) as reported in our study. In some rare cases,
366 O_2 consumption was reported to increase under high pCO_2 conditions (Wood *et al*., 2010;
367 Cummings *et al*., 2011). The effects of high CO_2 concentrations on metabolism appear
368 species-specific and depend on resistance capacities of the organisms (Melzner *et al*., 2009).
369 It has been widely reported that exposure to environmental high pCO_2 levels leads to changes
370 in homeostasis and extracellular acid-base balance counterbalanced by metabolic depression
371 in many cases (Pörtner *et al*., 2005; Pörtner, 2008), although it should be noted, as above, that
372 metabolic depression is often not seen in high pCO_2 conditions. Differences in acid-base
373 regulatory capacities by increasing HCO_3^- internal concentrations (Michaelidis *et al*., 2005;
374 Gutowska *et al*., 2010) or H^+ excretion (Pörtner *et al*., 2005) are taxon specific and are more
375 or less effective in mitigating the effects of hypercapnia. It has also been suggested that
376 organisms could maintain low metabolic rates without controlling internal pH by not using
377 pH-sensitive oxygen-binding pigments (Thomsen *et al*., 2010; Hiebenthal *et al*., 2013). Such
378 mechanisms may be crucial factors in explaining the observed variation in sensitivities and
379 resistances of marine invertebrates to elevated pCO_2 conditions (Gutowska *et al*., 2010).

It is important to note here that many of the studies to date on the effects of elevated pCO_2 on organisms are short-term and acute (e.g. Tomanek *et al*., 2011), not reflecting the long-term trade off in energy balance and physiological changes associated with acclimation of new environmental conditions (Clark *et al*., 2013). For example, metabolic depression acts as a time-limited compensation strategy to survive unfavorable condition such as high CO_2 concentrations (Guppy & Withers, 1999; Willson & Burnett, 2000). Because *C. fornicata* were held for 10 weeks in the different pCO_2 treatments in this investigation, it is likely there was enough time for them to acclimate to the new pH, and no difference in oxygen consumption was detected between the different pCO_2 conditions. However, the energetic cost likely produced by the negative effects of elevated pCO_2 may either be relatively small , or difficult to maintain over longer time periods. This could be seen in impacts on other physiological processes than respiration (Catarino *et al*., 2012). For example, Bibby *et al*., (2008) demonstrated that exposure to hypercapnic conditions may compromise the ability to express an immune response in mussels. They showed that *Mytilus edulis* phagocytosis declined as function of decreased pH. In the same way, Matozzo *et al*., (2012) showed that elevated pCO_2 and temperature may strongly affect haemocyte functionality in the bivalves *Chamelea gallina* and *Mytilus galloprovincialis*. Other cellular processes have also been shown to be negatively impacted by high CO_2 concentrations, including protein synthesis in the sipunculid *Sipunculus nudus* (Langenbuch *et al*., 2006) or enzyme activities in *C. gallina* and *M. galloprovincialis* (Matozzo *et al*., 2013). However, studies of the impact of reduced pH on immune systems have generally been of short duration and it would be interesting to investigate other physiological parameters than respiration (e.g. calcification, protein production, immunity regulation, fertility) in *C. fornicata* acclimated over several months in the different pCO_2 conditions predicted for the end of the century. As a coastal species adapted to relatively large fluctuations of abiotic parameters, *C. fornicata* in this study were

strongly resistant to both elevated pCO_2 and increased temperature. Indeed, resistance to high pCO_2 levels can also come from pre-acclimation or pre-adaptation to fluctuations in the environment where species live (Burnett, 1997). Species living in environments with large abiotic variation have a high phenotypic plasticity which can allow them to survive in stressful conditions (Hofmann & Todgham, 2010). Coastal organisms are more exposed to physico-chemical variations than their open-ocean counterparts that live in more stable thermal and pH environments (Berge *et al*., 2006; Peck *et al*., 2006). Species living in shallow waters tolerate not only seasonal and extreme temperature events but also periodic large fluctuations in seawater pH, driven by biological process that sequester and release large amounts of CO_2 (Beniash *et al*., 2010). This exposure to a wide environmental variation has likely led to the evolution of resistance mechanisms to abiotic factors including variations in pCO_2 and/or pH (Lannig *et al*., 2010).

C. fornicata is an invasive species which has successfully colonized European coastal shallow waters. This species is likely to have high phenotypic plasticity and resilience to physico-chemical variations that determined its success. Indeed, successful invasive species generally share characteristics that allow them to establish, colonize and expand their range. Among these characteristics, tolerance to environmental stress is one of the most common (Lenz *et al*., 2011). In a global change context, the movement of physico-chemical conditions away from the optimum increases the energy required by marine species to fuel the extra processes entrained to resist the stresses involved and to maintain homesostasis. This may result in changes in overall physiological condition (Cummings *et al*., 2011) that could impact ecological processes and community interactions. The high resilience to altered pCO_2/low pH levels observed here for *C. fornicata* may confer a competitive advantage to this invasive species over taxonomically or functionally related species (Lenz *et al*., 2011). For example, the performance of the scallop *Pecten maximus,* which is one of the *C. fornicata* competitors

(Thouzeau *et al*., 2000; Fresard & Boncoeur, 2006), has been shown to be negatively affected by high pCO_2 levels (Schalkhausser *et al*., 2013). These different sensitivities to environmental factors will likely dictate "winners" and "losers" among marine species that could lead to a restructuring of benthic communities. With other studies, our data suggest this restructuring could favor invasive species as evidence is building that shows they are more resistant to change than their native competitors (Dukes & Mooney, 1999; Occhipinti-Ambrogi, 2007).

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FIGURES CAPTIONS

Figure 1: Cumulated mortalities during the temperature increase. Males are represented on the graph on the top and females are on the graph in the bottom. The greyscale represent the different pCO_2 levels in which *C. fornicata* individuals where held during the experiment.

Figure 2: Mean (± SE) conditions indices at the beginning (black bar), and at the end of the experiment for *C. fornicata* females (white bars) and males (grey bars) in the different pCO_2. $27 > N > 10$

Figure 3: Respiration rates as a function of increasing temperature in each pCO_2 treatment, for *C. fornicata* males (top, triangles) and females (bottom, circles). Detailed statistical analyses relative to the regressions can be found in Table 3.

Figure 4: Mean (± SE) Q_{10} values for *C. fornicata* females (white bars) and males (grey bars) in the different pCO_2 treatments. $N = 3$

TABLES

Table 1: Mean (± standard error, SE) carbonate chemistry parameters for each pCO_2 treatment. pH (on the total scale, pH_T) was measured daily and total alkalinity (A_T) was measured every 3 weeks. Other parameters were calculated with CO2sys software. pCO_2 : CO_2 partial pressure; Ω_{Ar} : saturation state of seawater with respect to aragonite.

pCO_2 treatment	pH_T	pCO_2 (µatm)	Ω_{Ar}	A_T (µEq kg^{-1} SW)
	n = 69	n = 69	n = 69	n = 76
380 µatm	8.13 ± *0.01*	324 ± *8*	2.72 ± *0.06*	2333 ± *1*
550 µatm	7.89 ± *0.01*	619 ± *16*	1.69 ± *0.04*	2334 ± *2*
750 µatm	7.75 ± *0.01*	873 ± *20*	1.28 ± *0.03*	2335 ± *2*
1000 µatm	7.66 ± *0.01*	1138 ± *65*	1.05 ± *0.02*	2334 ± *2*

Table 2: Summary of two-way ANOVAs testing the effects of pCO_2, sex and the interaction of these two factors on the final condition indices (CI) and the Q_{10} values determined for *C. fornicata* males and females in the different pCO_2 conditions (380, 550, 750 and 1000 µatm). Bold numbers indicate significant level greater than 95%.

		CI		**Q_{10}**	
	df	F-value	p-value	F-value	p-value
pCO_2	3	1.245	0.295	0.657	0.590
sex	1	2.472	0.118	6.124	**0.025**
pCO_2 x sex	3	1.371	0.254	2.293	0.117

792 **Table 3**: Relationships between *C. fornicata* male and female respiration rates and

793 temperature in each pCO_2 treatment

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	pCO_2	Regression equation	n	R	R^2	F	p
males	380	y = 3.691 x - 34.455	42	0.60	0.37	22.97	< 0.001
	550	y = 2.993 x - 18.461	42	0.46	0.21	10.56	0.002
	750	y = 2.406 x - 4.543	41	0.40	0.16	7.55	0.009
	1000	y= 3.701 x - 41.556	41	0.56	0.31	17.37	< 0.001
females	380	y = 1.826 x - 7.635	42	0.49	0.24	12.72	< 0.001
	550	y = 1.585 x - 4.218	42	0.55	0.30	16.89	< 0.001
	750	y = 2.637 x - 26.240	42	0.63	0.40	26.66	< 0.001
	1000	y = 1.442 x + 3.435	42	0.37	0.14	6.26	0.017

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